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A Hybrid Solid State NMR and Electron Microscopy Structure Determination Protocol for Engineering Advanced Paracrystalline Optical Materials

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Abstract: We demonstrate hybrid MAS NMR and TEM for *de novo* structure determination of para-crystalline materials with a bioinspired fused naphthalene diimide (NDI) - salphen - phenazine prototype light harvesting compound. Starting from chiral building blocks with C_2 molecular symmetry we determine the asymmetric unit by MAS NMR, index low resolution TEM diffraction data, resolve reflection conditions, and show for the first time ability to determine the space group from reciprocal space data using our hybrid approach. Transfer of molecular C_2 symmetry into $P2_1/c$ packing symmetry provides a connection across length scales to overcome both lack of long range order and missing diffraction phase information. Refinement with heteronuclear distance constraints confirms the racemic $P2_1/c$ packing that is scaffolded by molecular recognition of salphen zinc in a pseudo-octahedral environment with bromide, and with alkyl chains folding along the phenazine. The NDI light harvesting stacks run orthogonal to the intermolecular electric dipole moment present in the solid. Finally, we determine the orientation of flexible lamellae on an electrode surface.

Chemical self-assembly to bridge the gap from dead to living matter is a challenging field. Controlling complexity, flexibility and functionality of synthetic and biomimetic material requires^[1] engineering soft para-crystalline compounds that lack crystalline long range order in at least one dimension. However, resolving their microscopic and mesoscopic order at atomic resolution from the anisotropic background heterogeneity has proven to be quite difficult. Only recently has the hybrid bottom up MAS NMR and top-down EM structure determination methodology shown promise to overcome the limitations of either technique.^[2] MAS NMR is intrinsically a microscopic method^[3] that cannot solve a structure *de novo* as packing order is determined by minimizing steric hindrance with screw axes or glide planes at higher levels

in the structural hierarchy. For small organic molecules forming microcrystals, modeling protocols are used with a conjectured rather than determined space group, making MAS NMR a chemical shift filter for selection and validation.

Earlier we showed for the largest biological (protein-free and grossly heterogeneous) light antennae that the limitations of MAS NMR can be diminished by using a 2D TEM periodogram as a band pass filter to resolve sparse regions of nonzero intensity in reciprocal space.^[4] Here we present the important next step by showing that genuine space group information can be extracted from TEM data in reciprocal space.

To demonstrate this we use a prototypical engineered biomimetic chromophore light-harvesting material that consists of fused Br-substituted naphthalene diimide (NDI), phenazine and Zn-salphen building blocks (Figure 1).^[5] This system, denoted as DATZnS(3'-NMe), models the chlorosome that is built from parallel stacks of bacteriochlorophyll (BChl) connected by metal coordination in a recognition motif. The BChl stacks form polarizable curved sheets and tubes that are thought to yield a dielectric response upon excitation with crossing of energy levels and coherent mixing of exciton states for energy transport. Although the model is chemically unrelated to BChl, we show that it self-assembles into stacks that form extended polar curved sheets like the natural paradigm. The NDI of the model is capable of overlap, the phenazine carries the electric dipole, and the Zn-salphen provides a recognition motif for coordination.^[6]

The molecular symmetry and asymmetric unit were determined from MAS NMR shifts collected with ^{13}C at natural abundance. We indexed the TEM diffraction and determined both the unit cell parameters and a genuine space group by analysis of systematic absences in a sparse reciprocal space

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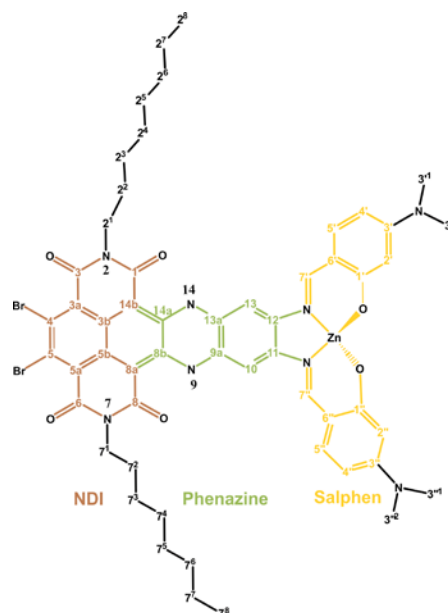


Figure 1. Chemical structure of *anti* DATZnS(3'-NMe), a fused hybrid of NDI (red), Phenazine (green) and Salphen (yellow).

intensity pattern with the help of the molecular symmetry information obtained by MAS NMR. The technologies were then merged at the molecular level and unit cell. As indexing of an

unknown structure is not possible by TEM alone, the diffraction phase problem was implicitly overcome by connecting across

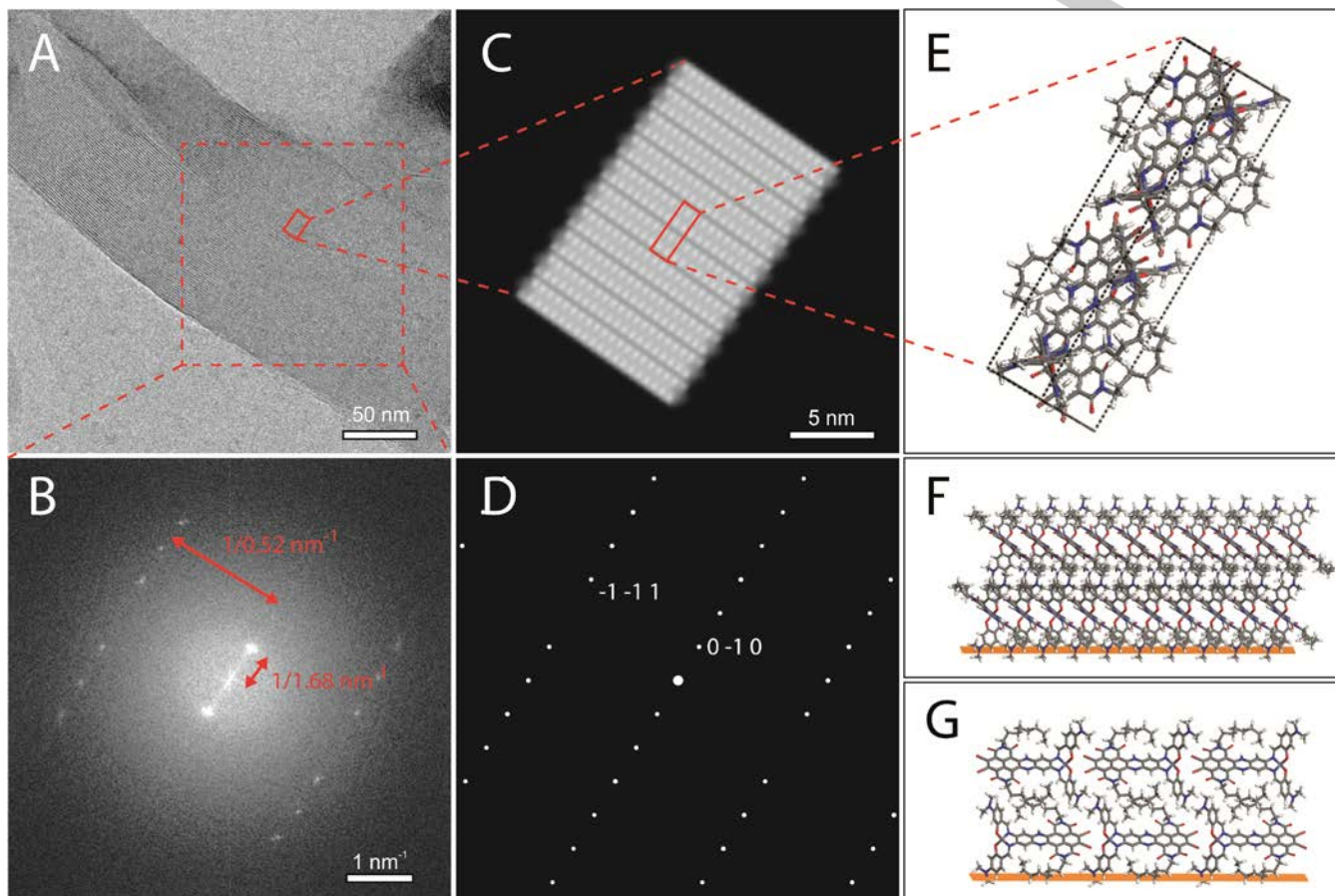


Figure 2. Integration of TEM and MAS NMR. (A) TEM image of the DATZnS(3'-NMe) on a carbon coated grid, revealing the curved lamellar character of the compound, (B) Fourier transform of the selected region showing the TEM diffraction pattern with systematic absences, (C) projection of the electron density map with a $20 \times 10 \times 5$ supercell,^[8] and (D) simulated diffraction pattern obtained with the NMR derived geometry in the $P2/c$ space group.^[9] (E) Orientation of DATZnS(3'-NMe) on the carbon grid (orange color), (F) viewed along the 'b' axis, and (G) along the 'a' axis.

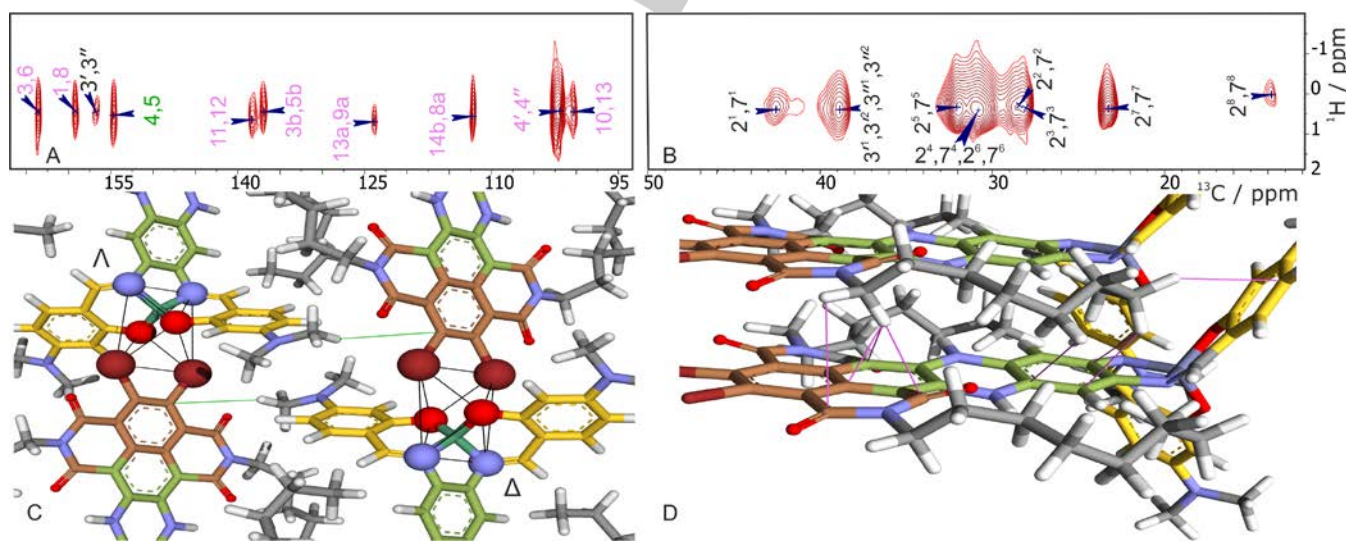


Figure 3. (A,B) Contour plot sections of ^1H - ^{13}C correlation spectra collected from DATZnS(3'-NMe) with a contact time of 4 ms. (C) The intermolecular interaction (green) between $4/5$ ^{13}C and dimethyl amine and the molecular recognition motif. (D) The folding of the tails is obtained from the transfer (violet) between phenazine ^{13}C nuclei and the aliphatic ^1H of the tails.

length scales through transfer of molecular symmetry to packing symmetry. This provides a structural underpinning for engineering supramolecular material in the desired sheets with parallel aligned dipoles. Fourier transform filtering in reciprocal space averages static heterogeneity in real space and enables extrapolating the short and medium range ordering in a paracrystalline lattice to establish a full 3D lattice model which we refined with MAS NMR heteronuclear ^1H - ^{13}C correlation data. Finally, we determined the orientation of DATZnS(3'-NMe) on a surface to relate to the geometry of biomimetic material in electrode applications.

The symmetric NMR response of the DATZnS(3'-NMe) provided conclusive evidence that the asymmetric unit is half of the molecule (Figure S1, Table S1). Of the two possible configurations, the *syn* form with a mirror plane running along the center of the phenazine motif is ~ 10 kcal/mole less stable than the *anti* form that has a twofold axis.^[7] This is the first step in our approach.

High-resolution TEM of the system on a carbon grid revealed curved lamellae (Figure 2A). The Fourier transform showed strong centrosymmetric reflections at $1/1.685\text{ nm}^{-1}$ and perpendicularly a series of spots at $1/0.547\text{ nm}^{-1}$ with a systematic absence indicating $h0l$ ($l = 2n$) reflections (Figure 2B). Both strong features are attributed to first order reflections and point to the molecule along the 1.685 nm direction with its C_2 axis representing $P2$ packing symmetry. The systematic absence reveals an additional translation with a screw axis or glide plane. A screw in a different direction would imply an orthorhombic cell, in contrast with EM images that reveal a monoclinic cell with intensity at $1/1.24\text{ nm}^{-1}$ that is attributed to second order reflections for a realistic density of 1.67 g/cm^2 (Figure S5). This leaves a glide plane that explains both the systematic absence at $1/0.547\text{ nm}^{-1}$ in Figure 2 and the absence of first order reflections in Figure S5. The mirror operation produced a racemic packing with the wings of the salphen forming enantiomeric chiral Λ and Δ pairs.^[10] This leads to a $P2/c$ space group with four inequivalent sites, two from the twofold axis in the DATZnS(3'-NMe) and two from the enantiomeric pair. This represents the second step of our approach, where we have overcome the diffraction phase problem and index the TEM data to resolve a genuine space group. Considering the weakness of the reflection spots in Figure S5 there can be other polymorphs as well, but these do not pass the TEM diffraction filter.

The $P2/c$ structure was modeled with unit cell dimensions $a = 0.547\text{ nm}$, $b = 1.685\text{ nm}$, $c = 2.517\text{ nm}$ and $\beta = 102^\circ$, determined with TEM to an energy of 170.5 kcal/mol (see S10 for details). To validate the structure, we performed an optimization without constraining the cell, which led to virtually the same result. Next, long range transfer signals were identified in a heteronuclear ^1H - ^{13}C dataset recorded with a long contact time of 4 ms by comparing with data collected for a short mixing time (Figure S3) and observed between the $3'^1$, $3'^2$, $3''^1$, $3''^2$ protons of salphen and $4, 5\text{ }^{13}\text{C}$ nuclei in the NDI motif (Figure 3). Transfer involving 3'-NMe and $4, 5\text{ }^{13}\text{C}$ provides strong NMR evidence for molecular recognition between the NDI part of the molecule and the salphen motif of an adjacent molecule. Transfer of polarization between protons on the alkyl chain and the $11, 12, 3b, 5b, 13a, 9a, 14b, 8a, 10, 13\text{ }^{13}\text{C}$ nuclei on the phenazine backbone positions the alkyl chain in the packing. The buildup of LGCP signals for the $4, 5$ and $13a, 9a\text{ }^{13}\text{C}$ from Figure S4 is in line with a simulation of transfer over $\sim 4\text{ \AA}$. (Figure 3).^[11] The $3b, 5b\text{ }^{13}\text{C}$ in the central part of the NDI motif correlate with the 2^1 and 7^1 CH_2 protons. This reveals formation of slipped J -aggregates for the NDI, with the alkyl tail above the plane of a neighbouring molecule. It implies that the correlations and buildup from protons at the alkyl chain to $13a, 9a\text{ }^{13}\text{C}$ are also

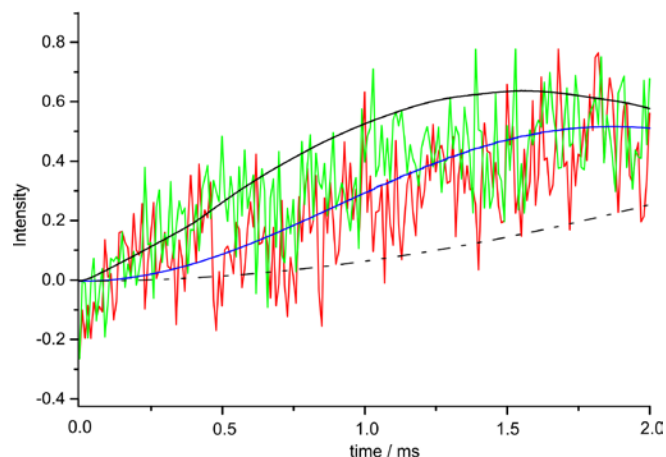


Figure 4. LG-CP build-up curves of the polarisation transfer to 4/5 (red) and 13a/9a (green) carbon nuclei compared with a simulated buildup for a heteronuclear ^1H - ^{13}C spin pair separated by $\sim 4\text{ \AA}$ (blue). The build up curves representing 3 \AA (black solid lines) and 5 \AA (black dotted lines) are also shown

intermolecular. Similarly, buildup of CP intensity from the alkyl CH_2 to the quaternary ^{13}C on the phenazine core can be considered intermolecular from the abundant cloud of protons on the alkyl chain.

Strong π - π stacking interactions and aligned electric dipoles explain why the material has a high density and a low energy and is insoluble. The aliphatic tails are oriented in the same direction as the salphen wings and are in voids between the phenazine moieties. Tight packing with the alkyl chains folded along the phenazine bridge of a neighbouring molecule explains the observation of strong heteronuclear correlation signals between the abundant aliphatic ^1H and rare phenazine ^{13}C spins providing efficient pathways for polarization transfer (Figure 3). The molecular recognition and distorted octahedral surrounding of the Zn^{2+} ion puts the 3'-NMe of salphen at 0.45 nm from the $4, 5\text{ }^{13}\text{C}$ in NDI, which is in quantitative agreement with the LGCP buildup kinetics and its simulation. This refinement represents the third step in our approach.

In our final and fourth step we determined the orientation of the material on the surface by simulating the diffraction pattern, thereby validating the indexing and space group. A view along the $0.69, 0, 0.69$ lattice vector gave the best match for the density and the diffraction pattern (Figure 4 C,D).^[9] The analysis validated the systematic absence of reflections from the c -glide plane in the $P2/c$ space group, and showed that $-1\ 0\ 1$ and $1\ 0\ -1$ are quenched (Figure 4B). The strong $0\ 1\ 0$ and $0\ -1\ 0$ are from lamellar spacing and alternating regions of Zn-salphen and NDI. The phenazine dipoles are aligned along the surface and are perpendicular to the NDI stacks that run parallel to the surface with the plane of the NDI rings at an angle of 45° (Figures 4E, F). Figure 5 shows how molecular recognition leads to transfer of molecular symmetry for scaffolding. Whereas steric hindrance favors screw axes or glide planes (symmetry operations with a translational component) to allow for interpenetration of symmetry-related molecules, the screw axis is apparently suppressed in DATZnS(3'-NMe) in favour of a twofold axis to accommodate intramolecular C_2 symmetry (Figure 5A). This is possible because of the rich structural variability introduced with the nonplanar metal salphen. It allows for packing in an achiral $P2/c$ space group with a racemic mixture of the two enantiomeric species, thereby circumventing the need for a screw axis in favour of a c -glide plane with inversion symmetry in the structure.

With C_2 molecular symmetry preserved, DATZnS(3'-NMe) self-assembles into polar planes without inversion symmetry

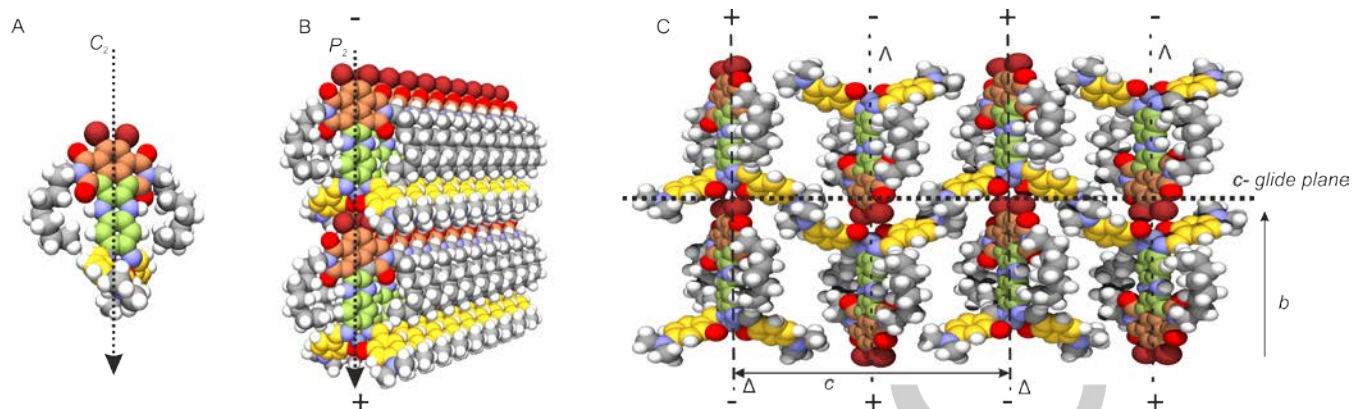


Figure 5. High resolution 3D lattice model for para-crystalline DATZnS(3'-NMe). (A) Molecular recognition for steering the packing starts from chiral building blocks with C_2 symmetry. (B) These self-assemble into enantiomerically pure Δ and Λ polar layers with a transfer of molecular C_2 symmetry into supramolecular P_2 symmetry. The layers comprise arrays of aligned dipoles with a positively charged salphen and negatively charged Br. (C) Alternating layers with opposite chirality self-assemble with a c -glide plane to release steric hindrance and establish dense a packing with quenching of electric dipoles.

elements, thereby mimicking the parallel stacking in the chlorosome antenna (Figure 5B) where the selectivity induced by chirality emerges at the salphen motif. The electric dipoles align and form extended arrays with a positive and a negative side to support charge separation following light absorption in the NDI columns running perpendicular to the electric field direction (Figure 5). DATZnS(3'-NMe) forms in the proposed 3D model extended chiral layers arising from planar arrangements of individual C_2 motifs, (known as organizational chirality) in which the net dipole moment is canceled due to antiparallel layers.

In conclusion, we demonstrate hybrid MAS NMR and TEM for *de novo* structure determination of a bioinspired para-crystalline material. The concept can be further developed with *e.g.* pattern recognition across TEM and NMR datasets to facilitate the applicability and broaden the scope. This paves the way for structure determination of advanced organic supramolecular materials that bridge the gap from dead to living matter and are inaccessible to high resolution diffraction methods.

Acknowledgements

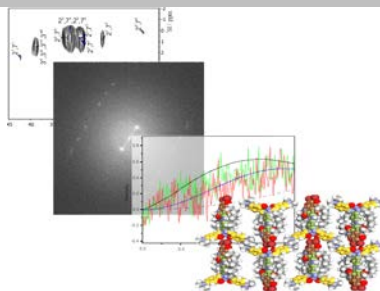
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Keywords: NMR spectroscopy; Electron microscopy; Photochemistry; Molecular recognition; Self-assembly; Magic Angle Spinning; Artificial Photosynthesis

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COMMUNICATION

Packing of a fused chromophore light harvesting material is resolved with a four-step hybrid approach involving MAS NMR and cryo-EM. Chemical shift and distance constraints from NMR data are combined with reflection conditions from the Fourier transform of EM images to overcome heterogeneity and problems with indexing the diffraction and determining the spacegroup.



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